Invasive aspergillosis induces complex chemokine and cytokine responses

Subjects: Infectious Diseases

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Invasive aspergillosis is a frequent complication in immunocompromised individuals, and it continues to be an important cause of mortality in patients undergoing hematopoietic stem cell transplantation. In addition to antifungal therapy used for mycoses, immune-modulatory molecules such as cytokines and chemokines can modify the host immune response and exhibit a promising form of antimicrobial therapeutics to combat invasive fungal diseases. Cytokine and chemokine profiles may also be applied as biomarkers during fungal infections and clinical research has demonstrated different activation patterns of cytokines in invasive mycoses such as aspergillosis.

Keywords: chemokines ; Aspergillus ; hematopoietic stem cell transplantation ; cytokines

1. Background

Fungi are among the most extensively distributed microorganisms and are ubiquitous in the environment. However, a small percentage of these remarkable eukaryotes are also major human pathogens. The frequency of opportunistic fungal infections continues to increase due to the expansion in the numbers of immunocompromised hosts [1]. Aspergillus species (spp.) are one of the most common medically important opportunistic fungi [2]. Invasive infections with Aspergillus spp. are typically considered life-threatening and most frequently occur in immunocompromised individuals such as those receiving chemotherapy, undergoing solid organ transplantation (SOT), or hematopoietic stem cell transplantation (HSCT) [3][4]. Among the human pathogenic species of the genus Aspergillus, Aspergillus fumigatus is the most common causative agent, followed by A. flavus, A. terreus, and A. niger [5]. In compromised hosts, Aspergillus infections most commonly manifest as invasive pulmonary aspergillosis (IPA). The number of patients undergoing transplantation has grown exponentially in recent years, particularly in patients undergoing HSCT for the treatment of hematological malignancy [6]. IPA occurs in 3.6 to 10.3% of allogeneic HSCT recipients leading to a mortality rate of 50 to 80% [6][7].

2. Cytokines and Chemokines Responses in Invasive Aspergillosis after Hematopoietic Stem Cell Transplantation

Cytokines and chemokines are biologically active secreted proteins released by immune cells that play critical roles in cell-to-cell communication. In aspergillosis, they are an important component of host defenses against infection by promoting the initiation, maintenance, and resolution of the host response [8]. Innate immune cells composed of granulocytes, monocytes, AECs, and DCs are the first line of defense against Aspergillus and are the cells that primarily combat the fungus within the first week after infection [8]. In addition, macrophages phagocytose Aspergillus conidia and inhibit their intracellular germination in the early phase of infection [9], which induces the expression of inflammatory chemokines and cytokines. Furthermore, neutrophils and circulating monocytes damage hyphae by secreting oxidative and non-oxidative microbicidal compounds [10]. Hence, early neutropenia followed by immunosuppressive drugs in HSCT leads to defects in certain immune-related phagocytosis. Thus, these findings indicate that the association between HSCT and the immune system is highly dynamic [11].

The results from in vitro analyses reveal that infection of the immature dendritic cells (iDCs) with small germinating conidia (approximate size, 3–8 µm) significantly increased the secretion of specific cytokines (IL-6, IL-12, TNF-α, and IL-10) and chemokines (IL-8, CCL20, and CXCL10) and the expression of immune receptors (PTX3, CXCR4, CCRL2, and IL2RA) [12]. The significant increase of both the pro-inflammatory cytokine TNF-α and chemoattraction chemokines IL-8, CCL-20, and CXCL10 were also observed with stimulation by the Aspergillus antigen 18-kDa RNase Aspf1 [13], compared to the levels expressed by unstimulated DCs.

Natural killer (NK) cells are lymphoid cells in peripheral blood that play a critical role in the innate host defense and their cell numbers are related to the severity of IPA [11]. NK cells are known for their release of cytokines and play a unique role

in the early phase of an immune response against Aspergillus [14]. In vitro infection of human NK cells by A. fumigatus hyphae for 6 h increases the secretion of inflammatory cytokines, such as IFN-γ, TNF-α, and growth factor GM-CSF, as well as several chemokines, including CXCL8/IL-8, CCL3/MIP-1α, CCL4/MIP-1β, and XCL1/lymphotactin [14]. Supporting the results from in vitro studies, a murine intranasal infection model using A. fumigatus conidia suggested that susceptibility to IA is associated with the levels of genes encoding IL-5 (a Th2 cytokine involved in B cell and eosinophil activation) and IL-17a (a Th17 inflammatory cytokine produced by T cells and NK cells). The increased expressions of the genes encoding IFN-γ, high levels of TNF-α and the upregulation of a network of TNF-α–related genes were significantly related to Aspergillus infection [15]. Additionally, the expression of classical Th2 cytokines (IL-4, IL-5, IL-13) was found in bronchiole epithelial lung homogenates of Aspergillus protease-induced murine inhalation model compared to the PBS-treated controls [15].

Several studies have demonstrated an alteration of cytokines and chemokines in patients with hematological malignancy undergoing HSCT who subsequently develop invasive fungal disease [16][17][18] and IA in particular [16][19][20]. For example, in adult hematology patients with proven/probable invasive fungal disease (IFD), increases of serum cytokine levels of IL-15 and IL-2R as well as chemokines levels of CCL2 and MIP-1α were observed, whereas the level of IL-4 was significantly lowered, compared to those with no evidence of IFD [16]. Another study in adult hematology patients with probable/possible IA reported higher levels of cytokine IL-6 and chemokine IL-8 in serum and significant elevations in bronchoalveolar lavage (BAL) fluid levels of IL-8, compared to those with other infections [19]. In support of these findings, Gonçalves et al. demonstrated that the BAL fluid levels of cytokines IL-1β, IL-6, IL-17A, IL-23, TNF-α, and chemokine IL-8 were increased in patients diagnosed with IA, which were also consistent with levels of these cytokines in serum [20]. Notably, although the expression of in vitro and in vivo cytokines/chemokines varies in the different studies, these discrepancies may be explained by differences in cell types responding to Aspergillus stimuli and the different patient populations. However, all these laboratory findings suggest that the elevation of cytokines/chemokines in serum and BAL fluid levels were associated with increased risk of IA and, thus, may be used as a valuable indicator of the risks associated with development of IA and guide enhanced antifungal prophylaxis and early treatment. These findings are summarized in **Table 1**.

Table 1. Cytokine and chemokine responses in invasive aspergillosis after hematopoietic stem cell transplantation.

Models	Samples	Methods	Major Findings			Interpretation
			Cytokines	Chemokines	Others	
In vitro						
iDC + A. fumigatus-small germinating conidia (6 h of stimulation)	**Infected iDCs**	**qRT-PCR**	↑ IL-6 ↑ IL-12 ↑ TNF-α ↑ IL-10	↑ IL-8 ↑ CCL20 ↑ CXCL10	↑ PTX3 ↑ TLR-2 ↓ TLR-4	**A. fumigatus germ tubes induced the expression of genes associated with recognition and phagocytosis in iDCs with a time-dependent manner.**
iDC + A. fumigatus antigen Aspf1	**Infected iDCs**	**qRT-PCR**	↑ TNF-α	↑ L-8 ↑ CXCL10 ↑ CCL20 ↑ CXCL8 /IL-8	• –	**Aspf1, a member of a family of conserved RNases, induces a pro-inflammatory cytokine response.**
NK cells obtained from PBMCs + A. fumigatus hyphae (6 h of stimulation)	**Infected NK cells**	**qRT-PCR**	↑ IFN-γ ↑ TNF-α ↑ GM-CSF	↑ CCL3/MIP-1α ↑ CCL4/MIP-1β ↑ XCL1/ lymphotactin	↓ NKp30 ↑ CD56	**NK cells reveal the expression and release of immunomodulatory molecules involved in antifungal immune responses.**

Models	Samples	Methods	Major Findings			Interpretation
			Cytokines	Chemokines	Others	
In vitro						
			In vivo			
Mice CD1 strain infected by intranasal instillation with A. fumigatus conidia (N = 24)	**Mouse whole-lung homogenates**	• qRT-PCR • ELISA	**Immunocompetent mice: Infected vs. Saline controls** ↑ IL-17a mRNA ↑ TNF-α protein level **Immunosuppressed mice: Infected vs. Saline controls** ↑ IFN-γ mRNA ↑ IL-17a mRNA ↑ TNF-α protein level ↑ IL-5 protein level **Immunocompetent vs. Immunosuppressed mice** ↓ TNF-α ↓ IFN-γ ↓ IL-4 ↓ IL-12p40			**Susceptibility to IA is associated with a high level of TNF-α at the site of infection and the upregulation of a network of TNF-α–related genes.**
BALB/c mice infected by intranasal instillation with A. fumigatus proteases, Aspf5 and Aspf13 (N = 20)	**Mouse lung homogenates**	• ELISA	**Infected vs. PBS controls** ↑ IL-4 ↑ Serum IgE ↑ IL-5 ↑ IL-13			**A. fumigatus secreted allergen proteases, Aspf5 and Aspf13, are important for induction of Th2 cytokines secretion and increased IgE levels, which are fundamental features of allergic asthma and an indication of disease severity.**
			Clinical study			
Adult hematology patients with proven/probable IFD (N = 172)	**Serum**	**ELISA**	↑ IL-15 ↑ IL-2R ↓ IL-4	↑ CCL2 ↑ MIP-1α	–	**High IL-2R and CCL2 concentrations as indicators for the risk of developing IFD.**
Adult hematology patients with probable/possible IA (N = 43)	• BAL • Serum	**ELISA**	**Serum** ↑ IL-6	**BAL** ↑ IL-8 **Serum** ↑ IL-8	↑ Aspergillus-specific lateral-flow device test	**High serum IL-8 levels were highly specific and highly sensitive for the diagnosis of IA.**

Models	Samples	Methods	Major Findings			Interpretation
			Cytokines	Chemokines	Others	
In vitro						
Patients diagnosed with IA (N = 48)	• BAL • Serum	**ELISA**	**BAL** ↑ IL-1β ↑ IL-6 ↑ IL-17A ↑ IL-23 ↑ TNF-α **Serum** ↑ IL-6 ↑ IL-17A ↑ IL-23	**BAL** ↑ IL-8 **Serum** ↑ IL-8	↑ Galactomannan in BAL specimens	**Alveolar cytokines might be useful in supporting current diagnostic approaches for IPA biomarkers.** **IL-8 was the best performing analyte with the most relevant discriminator between cases of IPA and controls.**

Abbreviations: BAL, bronchoalveolar lavage; CCL, chemokine (C-C motif) ligand; CD, cluster of differentiation; CXCL, chemokine (CXC motif) ligand; ELISA, enzyme-linked immunosorbent assay; GM-CSF, granulocyte-macrophage colony-stimulating factor; h, hour; IA, invasive aspergillosis; iDCs, immature dendritic cells; IFD, invasive fungal disease; IFN, interferon; IgE, immunoglobulin E; IL, interleukin; IPA, invasive pulmonary aspergillosis; MIP, macrophage inflammatory proteins; mRNA, messenger RNA; NK cell, natural killer cell; PBMCs, peripheral blood mononuclear cells; PBS, phosphate buffered saline; PTX, paclitaxel; qRT-PCR, quantitative reverse transcription polymerase chain reaction; TLR, toll-like receptor; TNF, tumor necrosis factor. The black arrows indicate the increase or decrease in cytokines and chemokines.

3. Genetic Polymorphisms in Hematopoietic Stem Cell Transplantation Patients Associated with Invasive Aspergillosis

Given the variable risk of infection and its clinical outcome among patients with comparable predisposing factors, genetic predisposition is considered as the most important factor of individual susceptibility to IA [21]. Aspergillus conidia or hyphae interact with the innate immune system through PRRs, which included Dectin-1, TLR-2, and TLR-4 [22]. These TLRs can trigger PI3K, MAPK, and ERK1/2 signaling pathways, resulting in the production of several cytokines and chemokines including IL-8, IL-1α, IL-1β, IL-17, TNF-α, CCL3, CCL4, as well as CXCL1 from immune cells [23][24]. Dectin-1 is an NK-cell-receptor-like C-type lectin that is widely expressed on monocytes, macrophages, DCs, neutrophils, and eosinophils [25]. Dectin-1 mediates antifungal immunity through the promotion of inflammatory activity, eventually leading to fungal clearance. Antifungal immunity can occur through the triggering of Syk, which leads to the induction of NF-κB and production of protective cytokine responses. Dectin-1 signals and induces the production of cytokines through a Syk-independent pathway (noncanonical NF-κB pathway) [26]. Therefore, genetic polymorphisms affecting human Dectin-1 can be partly attributed to defective cytokine production, leading to an increased susceptibility to IA. Defective production of TNF-α and IL-6 has been found in both PBMCs and BEAS-2B respiratory epithelial cells harboring the Dectin-1 Y238X polymorphism [27][28]. Additionally, Dectin-1 knockout in BALB/c mice have decreased production of IFN-γ, IL-17A, and IL-10, and have a significantly reduced ability to control Aspergillus infection [28]. Conversely, single nucleotide polymorphisms (SNPs) in the intracellular PRR NOD2 can decrease the risk of IA [22]. NOD2 deficiency results in a defective inflammatory response with alterations in the levels of IL-1β, IL-17A, IL-22, and IFN-γ produced by PBMCs from hematological patients undergoing allogeneic HSCT, and IL-6 and TNF levels in $Nod2^{-/-}$ deficient mice [29]. Furthermore, low levels of serum IL-10 and IL-8 have been reported in patients with hematological malignancies undergoing allogeneic HSCT [29]. Thus, targeting assays for alternations in NOD2 may be an attractive method in personalized management strategies for IA. However, at present, these findings fundamentally show that defects in NOD2 potentially reduce Aspergillus-induced cytokine driven inflammation. Importantly, it needs to be elucidated whether cytokine alterations mechanistically protect from fungal infection in HSCT patients with NOD2 variants. Furthermore, polymorphisms in other cytokine genes such as IL-1 and IL-10 have also been implicated as genetic biomarkers of susceptibility to IFD [30][31]. These findings are summarized in **Table 2**.

Table 2. Genetic polymorphisms in hematopoietic stem cell transplantation patients are associated with susceptibility/resistance to invasive aspergillosis.

Models	Polymorphism	Major Findings		Interpretation	Ref.
		Cytokines	Others		
In vitro					
PBMCs	**Dectin-1 Y238X Stop Codon Polymorphism + heat-killed A. fumigatus hyphae + live A. fumigatus conidia**	↓ TNF-α ↓ IL-6	↓ binding ability to β-glucan	**Dectin-1 Y238X resulted in the reduction of pro-inflammatory cytokines due to the Dectin-1 receptor, which is known to play a role in fungal cell wall β-glucan recognition.**	[40]
BEAS-2B (Respiratory epithelial cells)	**Dectin-1 blockade by siRNA + Stimuli (β-glucan or Aspergillus conidia)**	↓ IL-6 ↓ TNF-α	–	**Dectin-1 expressed on epithelial cells contributes to the production of cytokines.**	[41]
PBMCs from allogeneic HSCT	**NOD2 genetic variation - P268S (TT-genotype) + A. fumigatus conidia - complete NOD deficiency + A. fumigatus conidia**	**Infected in TT-genotype compared with infected in CC- and CT-genotype** ↓ IL-1β ↓ IL-17A **Aspergillus infected compared with uninfected** ↓ IL-1β ↓ IL-22 ↓ IFN-γ	**Aspergillus infected compared with uninfected** ↓ IL-17A$^+$, IL-22$^+$, and IFN-γ$^+$ CD4 T-cell populations	**Human NOD2 deficiency reduces Aspergillus-induced inflammatory cytokines.**	[42]
Human PMBCs from solid-organ transplant recipients	• IL1B rs16944 SNP + A. fumigatus conidia • IL1RN rs419598 SNP + A. fumigatus conidia	**IL1B rs16944 SNP** ↓ IL-1β ↓ TNF-α ↓ IL-22 **IL1RN rs419598 SNP** ↓ IL-1β ↓ TNF-α	• –	**Both IL1B rs16944 and IL1RN rs419598 SNPs effect Aspergillus-induced cytokine release.**	[43]
Macrophages from healthy blood donors	**IL10 SNP with GG genotype + A. fumigatus conidia**	↓ IL-10 ↓ TNF-α ↓ IL-6 ↓ IL-1β ↓ IL-8	↓ fungal clearance	**IL-10 overexpression influences IA by suppressing antifungal immunity.**	[44]
In vivo					

Models	Polymorphism	Major Findings		Interpretation	Ref.
		Cytokines	Others		
In vitro					
BALB/c mice with HSCT + Aspergillus (N = 16)	**Dectin-1 knockout mice**	↓ IFN-γ ↓ IL-17A ↓ IL-10	↑ fungal growth	**Dectin-1 modulates immunity and tolerance via IFN-γ / IL-10 production, and both cytokines activate the protection of Th1/Treg antifungal responses.**	**[41]**
Nod2-deficient ($Nod2^{-/-}$) C57BL/6 mice + Aspergillus (lethal dose) (N = 22)	**$Nod2^{-/-}$ deficient mice (Splenocytes)**	↓ IL-6 ↓ TNF	↑ 14-day survival	**NOD2 augments Aspergillus-induced cytokine responses and results in resistance to Aspergillus infection.**	**[42]**
Clinical study					
Patients who developed IA post HSCT (N = 71) Non-HSCT patients with IA (N = 21)	**Y238X Stop Codon Polymorphism**	• –	↑ susceptibility to IA	**Dectin-1 Y238X heterozygosity had a limited influence on susceptibility to IA.**	**[45]**
Hematological patients undergoing allogeneic HSCT (N = 310)	**NOD2 genetic variation - P268S SNP**	↓ serum IL-10 ↓ serum IL-8	↓ susceptibility to IA	**Genetic deficiency of NOD2 results in an alteration of cytokine production in response to Aspergillus infection.**	**[42]**
An allograft with IA (N = 81) or without IA (N = 58)	**CXCL10 genetic variation - C+11101T - C+1642G - A1101G**	↑ serum CXCL-10	↑ susceptibility to IA	**Polymorphisms in CXCL10 altered chemokine secretion and increased the risk of IA after alloSCT.**	**[40]**

Abbreviations: alloSCT, allogeneic stem cell transplantation; CXCL, chemokine (CXC motif) ligand; HSCT, hematopoietic stem cell transplant; IA, invasive aspergillosis; IFN, interferon; IL, interleukin; NOD, nucleotide oligomerization domain; PBMCs, peripheral blood mononuclear cells; SNP, single nucleotide polymorphism; Th cell, T helper cell; TNF, tumor necrosis factor. The black arrows indicate the increase or decrease in cytokines and chemokines. $Nod2^{-/-}$ indicates complete deletion (-) of Nod2 alleles.

References

1. Parisa Badiee; Zahra Hashemizadeh; Opportunistic invasive fungal infections: diagnosis & clinical management. *Indian Journal of Medical Research* **2014**, *139*, 195-204, .
2. Jeremy D. Weaver; Edward J. Mullaney; Xin Gen Lei; Altering the substrate specificity site of Aspergillus niger PhyB shifts the pH optimum to pH 3.2. *Applied Microbiology and Biotechnology* **2007**, *76*, 117-122, 10.1007/s00253-007-0975-z.
3. Jay A. Fishman; Infection in Solid-Organ Transplant Recipients. *New England Journal of Medicine* **2007**, *357*, 2601-2614, 10.1056/nejmra064928.
4. Claudia Stuehler; Esther Kuenzli; Veronika Jaeger; Veronika Baettig; Fabrizia Ferracin; Zarko Rajacic; Deborah Kaiser; Claudia Bernardini; Pascal Forrer; Maja Weisser; et al. Immune Reconstitution After Allogeneic Hematopoietic Stem Cell Transplantation and Association With Occurrence and Outcome of Invasive Aspergillosis. *The Journal of Infectious Diseases* **2015**, *212*, 959-967, 10.1093/infdis/jiv143.
5. J. Morgan; Kathleen Wannemuehler; K. A. Marr; S. Hadley; D. P. Kontoyiannis; T. J. Walsh; S. K. Fridkin; P. G. Pappas; D. W. Warnock; Incidence of invasive aspergillosis following hematopoietic stem cell and solid organ transplantation: interim results of a prospective multicenter surveillance program. *Medical Mycology* **2005**, *43*, 49-58, 10.1080/13693780400020113.

6. Nina Singh; David L. Paterson; Aspergillus Infections in Transplant Recipients. *Clinical Microbiology Reviews* **2005**, *18*, 44-69, 10.1128/cmr.18.1.44-69.2005.

7. Swu-Jane Lin; Jennifer Schranz; Steven M. Teutsch; Aspergillosis Case-Fatality Rate: Systematic Review of the Literature. *Clinical Infectious Diseases* **2001**, *32*, 358-366, 10.1086/318483.

8. Jan Storek; Michelle Geddes; Faisal Khan; Bertrand Huard; Claudine Helg; Yves Chalandon; Jakob R Passweg; Eddy Roosnek; Reconstitution of the immune system after hematopoietic stem cell transplantation in humans. *Seminars in Immunopathology* **2008**, *30*, 425-437, 10.1007/s00281-008-0132-5.

9. O. Ibrahim-Granet; B. Philippe; H. Boleti; E. Boisvieux-Ulrich; D. Grenet; Marc Stern; J. P. Latgé; Phagocytosis and Intracellular Fate of Aspergillus fumigatus Conidia in Alveolar Macrophages. *Infection and Immunity* **2003**, *71*, 891-903, 10.1128/iai.71.2.891-903.2003.

10. Richard D. Diamond; Robert A. Clark; Damage to Aspergillus fumigatus and Rhizopus oryzae Hyphae by Oxidative and Nonoxidative Microbicidal Products of Human Neutrophils In Vitro. *Infection and Immunity* **1982**, *38*, 487-495, .

11. Rohtesh S. Mehta; Katayoun Rezvani; Immune reconstitution post allogeneic transplant and the impact of immune recovery on the risk of infection. *Virulence* **2016**, *7*, 901-916, 10.1080/21505594.2016.1208866.

12. Markus Mezger; Susanne Kneitz; Iwona Wozniok; Oliver Kurzai; Hermann Einsele; Juergen Loeffler; Proinflammatory Response of Immature Human Dendritic Cells is Mediated by Dectin-1 after Exposure toAspergillus fumigatusGerm Tubes. *The Journal of Infectious Diseases* **2008**, *197*, 924-931, 10.1086/528694.

13. Michael Ok; Jean Paul Latgé; Carina Baeuerlein; Frank Ebel; Markus Mezger; Max Topp; Oliver Kurzai; Doreen Killian; Markus Kapp; Goetz-Ulrich Grigoleit; et al. Immune Responses of Human Immature Dendritic Cells Can Be Modulated by the Recombinant Aspergillus fumigatus Antigen Aspf1. *Clinical and Vaccine Immunology* **2009**, *16*, 1485-1492, 10.1128/cvi.00175-08.

14. Lothar Marischen; Anne Englert; Anna-Lena Schmitt; Hermann Einsele; Juergen Loeffler; Human NK cells adapt their immune response towards increasing multiplicities of infection of Aspergillus fumigatus. *BMC Immunology* **2018**, *19*, 1-12, 10.1186/s12865-018-0276-6.

15. Sara Namvar; Peter Warn; Edward Farnell; Mike Bromley; Marcin Fraczek; Paul Bowyer; Sarah Herrick; Aspergillus fumigatus proteases, Asp f 5 and Asp f 13, are essential for airway inflammation and remodelling in a murine inhalation model. *Clinical & Experimental Allergy* **2015**, *45*, 982-993, 10.1111/cea.12426.

16. M. Mansour Ceesay; Shahram Kordasti; Eamaan Rufaie; Nicholas Lea; Melvyn Smith; Jim Wade; Abdel Douiri; Ghulam J. Mufti; Antonio Pagliuca; Baseline cytokine profiling identifies novel risk factors for invasive fungal disease among haematology patients undergoing intensive chemotherapy or haematopoietic stem cell transplantation. *Journal of Infection* **2016**, *73*, 280-288, 10.1016/j.jinf.2016.04.040.

17. Kieren A. Marr; Fungal infections in hematopoietic stem cell transplant recipients. *Medical Mycology* **2008**, *46*, 293-302, 10.1080/13693780701885552.

18. Simon Eheidegger; Marcel R. M. Van Den Brink; Tobias Ehaas; Hendrik Epoeck; The Role of Pattern-Recognition Receptors in Graft-Versus-Host Disease and Graft-Versus-Leukemia after Allogeneic Stem Cell Transplantation. *Frontiers in Immunology* **2014**, *5*, 337, 10.3389/fimmu.2014.00337.

19. Sven Heldt; Juergen Prattes; Susanne Eigl; Birgit Spiess; Holger Flick; Jasmin Rabensteiner; Gemma Johnson; Florian Prüller; Albert Wölfler; Tobias Niedrist; et al. Diagnosis of invasive aspergillosis in hematological malignancy patients: Performance of cytokines, Asp LFD, and Aspergillus PCR in same day blood and bronchoalveolar lavage samples. *Journal of Infection* **2018**, *77*, 235-241, 10.1016/j.jinf.2018.05.001.

20. Samuel Gonçalves; Katrien Lagrou; Cláudia S. Rodrigues; Cláudia F. Campos; Leticia Bernal-Martínez; Fernando Rodrigues; Ricardo Silvestre; Laura Alcazar-Fuoli; Johan A. Maertens; Cristina Cunha; et al. Evaluation of Bronchoalveolar Lavage Fluid Cytokines as Biomarkers for Invasive Pulmonary Aspergillosis in At-Risk Patients. *Frontiers in Microbiology* **2017**, *8*, 2362-2362, 10.3389/fmicb.2017.02362.

21. Cristina Cunha; Franco Aversa; Luigina Romani; Agostinho Carvalho; Human Genetic Susceptibility to Invasive Aspergillosis. *PLOS Pathogens* **2013**, *9*, e1003434, 10.1371/journal.ppat.1003434.

22. Kathleen R. Bartemes; Hirohito Kita; Innate and adaptive immune responses to fungi in the airway. *Journal of Allergy and Clinical Immunology* **2018**, *142*, 353-363, 10.1016/j.jaci.2018.06.015.

23. Michelle Galeas-Pena; Nathaniel McLaughlin; Derek Pociask; The role of the innate immune system on pulmonary infections. *Biological Chemistry* **2018**, *400*, 443-456, 10.1515/hsz-2018-0304.

24. Fabio Re; Jack L. Strominger; Toll-like Receptor 2 (TLR2) and TLR4 Differentially Activate Human Dendritic Cells. *Journal of Biological Chemistry* **2001**, *276*, 37692-37699, 10.1074/jbc.m105927200.

25. Janet A. Willment; Hsi-Hsen Lin; Delyth M. Reid; Philip R. Taylor; David L. Williams; Simon Y. C. Wong; Siamon Gordon; Gordon D. Brown; Dectin-1 Expression and Function Are Enhanced on Alternatively Activated and GM-CSF-Treated Macrophages and Are Negatively Regulated by IL-10, Dexamethasone, and Lipopolysaccharide. *The Journal of Immunology* **2003**, *171*, 6297.3-6297, 10.4049/jimmunol.171.11.6297-b.
26. Sonja I. Gringhuis; Jeroen Den Dunnen; Manja Litjens; Michiel van der Vlist; Brigitte A Wevers; Sven C. M. Bruijns; Teunis B. H. Geijtenbeek; Dectin-1 directs T helper cell differentiation by controlling noncanonical NF-κB activation through Raf-1 and Syk. *Nature Immunology* **2009**, *10*, 203-213, 10.1038/ni.1692.
27. Markus Mezger; Michael Steffens; Melanie Beyer; Carolin Manger; Johannes Eberle; Mohammad-Reza Toliat; Thomas F. Wienker; Per Ljungman; Holger Hebart; Hans Jürgen Dornbusch; et al. Polymorphisms in the chemokine (C-X-C motif) ligand 10 are associated with invasive aspergillosis after allogeneic stem-cell transplantation and influence CXCL10 epression in monocyte-derived dendritic cells. *Blood* **2008**, *111*, 534-536, 10.1182/blood-2007-05-090928.
28. Cristina Cunha; Mauro Di Ianni; Silvia Bozza; Gloria Giovannini; Silvia Zagarella; Teresa Zelante; Carmen D'Angelo; Antonio Pierini; Lucia Pitzurra; Franca Falzetti; et al. Dectin-1 Y238X polymorphism associates with susceptibility to invasive aspergillosis in hematopoietic transplantation through impairment of both recipient- and donor-dependent mechanisms of antifungal immunity. *Blood* **2010**, *116*, 5394-5402, 10.1182/blood-2010-04-279307.
29. Mark S. Gresnigt; Cristina Cunha; Martin Jaeger; Samuel M. Gonçalves; R. K. Subbarao Malireddi; Anne Ammerdorffer; Rosalie Lubbers; Marije Oosting; Orhan Rasid; Grégory Jouvion; et al. Genetic deficiency of NOD2 confers resistance to invasive aspergillosis. *Nature Communications* **2018**, *9*, 2636, 10.1038/s41467-018-04912-3.
30. Agnieszka Wójtowicz; Mark Gresnigt; Thanh Lecompte; Stephanie Bibert; Oriol Manuel; L. A. B. Joosten; Sina Rüeger; Christoph Berger; Katia Boggian; Alexia Cusini; et al. IL1B and DEFB1 Polymorphisms Increase Susceptibility to Invasive Mold Infection After Solid-Organ Transplantation. *The Journal of Infectious Diseases* **2014**, *211*, 1646-1657, 10.1093/infdis/jiu636.
31. Cristina Cunha; Samuel Gonçalves; Cláudio Duarte-Oliveira; Luís Leite; Katrien Lagrou; António Marques; Carmen B. Lupiañez; Inês Mesquita; Joana Gaifem; Ana Margarida Barbosa; et al. IL-10 overexpression predisposes to invasive aspergillosis by suppressing antifungal immunity. *Journal of Allergy and Clinical Immunology* **2017**, *140*, 867-870.e9, 10.1016/j.jaci.2017.02.034.
32. Louis Y.A. Chai; Mark G. J. De Boer; Walter J. F. M. Van Der Velden; Theo Plantinga; Annemiek van Spriel; Cor Jacobs; Constantijn J. M. Halkes; Alieke G. Vonk; Nicole M. Blijlevens; Jaap T. Van Dissel; et al. The Y238X Stop Codon Polymorphism in the Human β-Glucan Receptor Dectin-1 and Susceptibility to Invasive Aspergillosis. *The Journal of Infectious Diseases* **2011**, *203*, 736-743, 10.1093/infdis/jiq102.